Perspective on classical vs biochemical views of gene
Description of new techniques in biochem genetics
Illustration of new approaches to genetic analysis

Science writers seminar March 28, 1981

Introduction: what is a gene? how does it work? what is the basis of mutation?

Classically: genes as "black boxes" (individible units of unknown composition) responsible for phenotypic trains; they segregate in crosses, are dominant and recessive

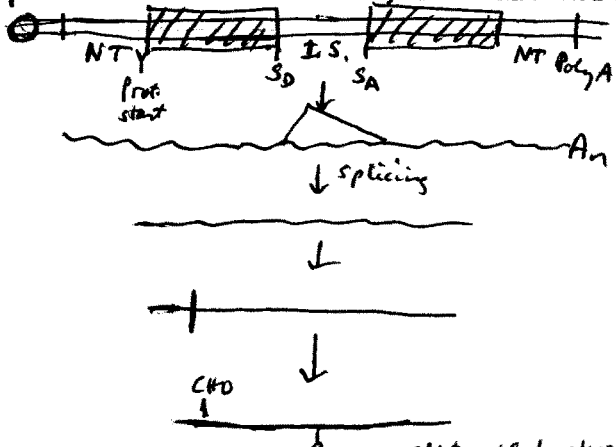
simple: gene → Phenotype
complex: ① →(A) ② →(B) ③ →(C) ④ phenotype CASCADE (pathway)
genes acting in concert Ⓐ Ⓑ Ⓒ → Phenotype REGULATION

Watson & Crick triggered revolution by proposing that genes are composed of DNA: biochemists became geneticists, central dogma laid down

ABCT TCGA AGCU

DNA → RNA → Protein

in simple form, DNA triplets encode amino acids; DNA transcribed to RNA translated to protein; mutations result from base substitutions (silent → no change / nonsense) deletion (frameshift, gene loss)



in current view, somewhat more complicated:

within scheme:
- genes are interrupted
- genetic unit includes regulatory signals: promoter, initation site, NT3' and 5', splice sites, poly Asignal.
- gene products (proteins) are further processed

beyond scope:
- genes are unstable (rearrangements, deletions, jumping genes; amplification)
- overlapping genes; use of 2 or 3 frames
- alternate splicing
- multigene families (complicates genetics df. Tom)
- mysterious DNA: introns, high copy repeats, endogenous viruses, unexpressed

modification of DNA → methylation, chromatin structure

Recent technical revolution has altered the prospects for analysis of genes--- epitomized by reverse genetics: CLONE (clone it, modify it, express it), i.e. make a desired alteration in a gene and then examine its effects; new techniques also applicable to biochemical study of conventional mutants, isolated on basis of phenotype.

Critical techniques: (expand as necessary)

molecular hybridization--allows detection of specific genes on the basis of nucleotide sequence rather than gene products

enzymatic manipulation---cutting, repairing, trimming, rejoining

fractionation of DNA by gel electrophoresis (+Southern transfer)

molecular cloning---allows gene isolation (chromosome fractionation)

principle: join DNA to molecule which can replicate in large numbers (gen. E.coli host, also yeast, mammalian cells)

need means of isolating or identifying gene of interest

- **---via mRNA
- ---viral DNA's (prepurified)
- ---select a volume from a library: cf. HGPRT + transformation assays
- ---make a DNA probe from protein sequence
- (others)

expression: introduction into living cells: transformation, euk. viral vect. microinjection

extensions:
- gene replacement in yeast
- new animals via teratoCa cells

RNA synthesis in extracts (+ polymerase)

DNA sequencing

DNA synthesis de novo

Putting many techs together: make mutations at selected sites in cloned DNA
reclone mutant genes
test for expression

(B) Using reverse genetics to examine regulatory signals: initiation of transcription

In bacteria, classical genetics produced large number of mutants proved to affect efficiency of transcription; many such mutants sequenced, and regulatory sequences thereby defined.

In higher eukaryotes, few genes for which selection techniques are available have been cloned and many of cloned genes are unlikely to produce selectable phenotype (e.g. lethal if not expressed; no effects, if member of repeated class; etc.). Reverse genetics particularly attractive in this context:
- transcriptional control testable both in cells and in test tube
- potential targets can be directly altered (and precisely)

disadvantages: hard work, neg. results less inform. than pos. results

Some examples: from sea urchin:

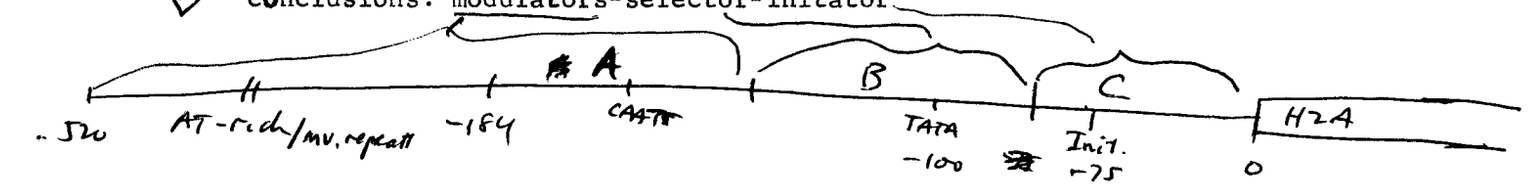
(1) Transcription of histone genes/ gene cluster, repeated genes; unlikely to be selectable cloning, sequencing and comparison with other genes indicates probable controls (illustrate)

make deletions and inversions and assay mutant DNA's after injection into oocyte nuclei from frogs(Xenopus)

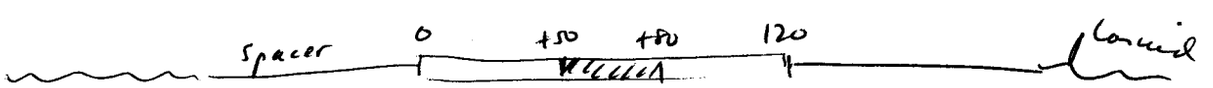
effects: del A---incr. freq of normal transcripts
del B---decr. freq and use of atyp. init. sites
del C---decr. freq and use of new site at expected distance from TATA

del AT rich region--marked decrease
invers " --4x increase plus additional start

conclusions: modulators-selector-initator



(2) Cloned 5S DNA from frog assayed in extract of frog oocyte nuclei
(again, repeated gene; no protein product in this case)
Note; this gene transcribed by a different RNA polymerase
Again make deletions, but result somewhat different:



? (3) LTR exptl.

Can produce abundant RNA of approx. correct size (sl. variation in start positions) as long as region of +50 to +80 left intact

Others - poly A signal mutants
splicing mutants (removes intrns, alters splice signals)

Prospects: isolation of more genes, esp. genes for enzymes
clearer concept of gene stability, mech. of mutation in mammals.
definition of regulatory elements (hormonal control, etc.)

Illustrate power of new tools in relation to two problems: transforming genes
transcriptional regulatory signals

re: viral replication / tumor viruses

(A) Describe viral genetics: phenotypic consequences of adding viral gene to cell

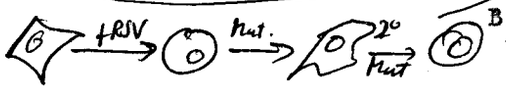
(src)

Illustrate with rat cell + RSV: one gene alters phenotype via production of 60K protein with kinase activity

identify mutants by return to normal phenotype

" secondary mutants by return to transformed phenot.

(1) How new techs. permit exploration of conventional mutants



Biochemical techniques permit: analysis of gene structure and position in cell chromosome

cloning of mutant genes and determination of nucleotide sequence

correlation with mutant protein (phosph., kinase act) and size with biol. effects.

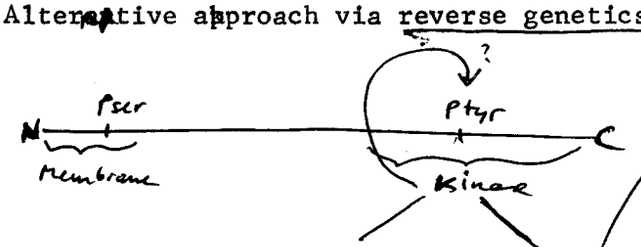
(2) Alternative approach via reverse genetics: clone wild type src gene

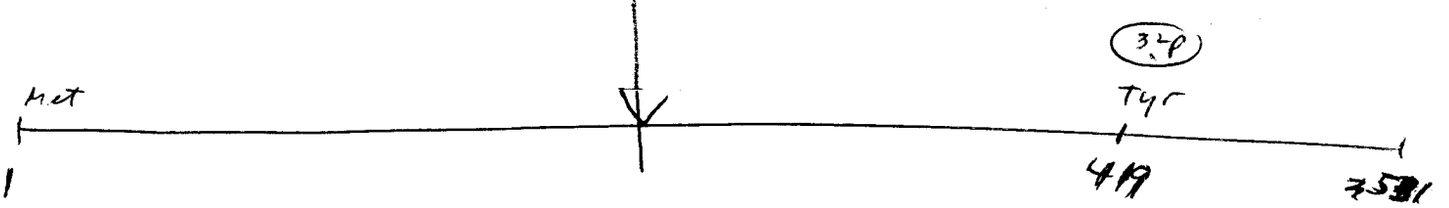
determine its sequence

"guess" at functionally interesting regions of protein

make mutations (deletions, point mutations) to affect these regions

put altered gene into cells (transformation in virus) + look for expression/effects





asp asn glu tyr thr ala arg

GAC AAC GAG TAC ACA GCA CGG

CTG TTG CTC TTG TGT

(ATG)

AAC

asn